Overview of the Public Interest Document

This document contains information to support the finding that the registration and exemption from the requirement of a tolerance for the plant -incorporated protectant DvSnf7 dsRNA and *Bacillus thuringiensis* Cry3Bb1 Protein, and the ge netic materials (Vector PV -ZMIR10871) necessary for their production in MON 87411 corn is in the public interest. This Public Interest Document has been prepared according to the guidelines issued by the EPA Office of Pesticide Programs entitled, "Conditional Registration of New Pesticides" (51 FR 7628 - 7634). These guidelines were specifically prepared to compare the benefits and risks of chemical pesticides, but are equally applicable to plant -incorporated protectants such a s Corn Rootworm (CRW) - protected corn. In the following discussion, we have addressed each of the applicable categories found under item IV Public Interest Finding.

Note to the Reviewer:

An application for FIFRA Section 3 seed increase registration of the plant-incorporated protectant, DvSnf7 dsRNA and *Bacillus thuringiensis* Cry3Bb1 protein and the genetic materials (Vector PV - ZMIR10871) necessary for their production in MON 87411 corn, comprising an administrative volume and 23 volumes of study findings (MRID Nos. 49315101-49315123), was submitted to EPA on February 4, 2014. The EPA file symbol for this product is 524-ARI.

Benefits and Public Interest Findings for Registration of Corn Event MON 87411

1. Introduction

Nucleic acids like deoxyribonucle ic acid (DNA) and ribonucleic acid (RNA) are found throughout the living world and are present everywhere in the environment. We are also exposed to these nucleic acids constantly through the foods we eat (Ivashuta et al. 2009; Trevors 1996) and the water we drink (Djikeng et al. 2009 ; Roossinck 2012). Almost all foods are derived from some kind of plant or animal source, and since all these sources contain nucleic acids, we routinely consume RNA and DNA on a daily basis. Everyday foods high in RNA content include organ meats, fresh seafood and dried vegetables (Carver and Walker 1995). In addition, since humans are exposed in nature to the same environment as plants and animals, there is also a clear lack of any hazard that could come from nucleic acids for humans or the environment.

One of the ways nucleic acids work is to control other nucleic acids and/or to control other things, like proteins. One of these pathways is a process that has to do with the regulation of a gene. This pathway is called RNA interference or RNAi, and is a naturally occurring process in plants (Brodersen and Voinnet 2006 ; Mallory and Vaucheret 2006) and animals (Dykxhoorn et al. 2003; Fire et al. 1998; Petrick et al. 2013; Siomi and Siomi 2009). RNAi is a natural process cells use to turn down, or suppress the activity of specific genes. RNAi works through the cell's natural ability to review RNA instructions inside the cell and then "decide" whether to turn down or stop product ion of a specific protein, much like a dimmer on a light switch. Specifically, RNAi works by naturally interfering with a cell's nucleic "messengers", called messenger RNA, that carry information to protein factories within the cell. If the factory doesn't receive the information, it can't produce the protein, essentially turning down or off the production of that particular protein. Importantly, RNAi is very specific , so that only the protein targeted at the factory is affected.

In addition, as a nucleic acid RNA is neither toxic, nor allergenic, and significant barriers exist in humans and other organisms that limit the uptake of RNA. The presence of these highly limiting barriers in people and animals makes sense, given living things naturally consume them constantly, and we have continued to coexist in their presence for millennia. This is true, despite the possibility that at least some of the multitude of naturally occurring RNAi may in theory target some of the proteins our factories are trying to produce.

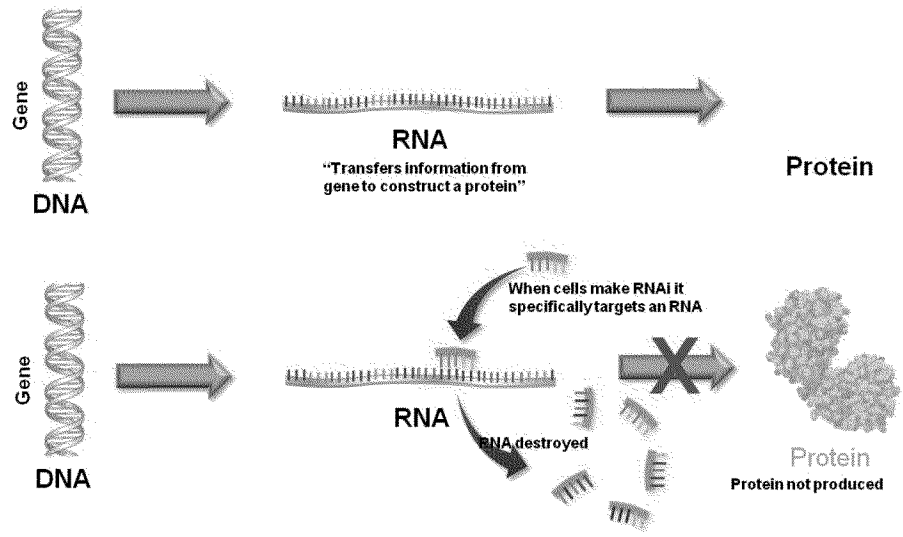

Figure 1. RNA interference (RNAi) is a natural process within a cell that can affect the production of proteins by controlling the expression of a gene.

The process was discovered in the 1990's by researchers attempting to enhance the purple color of petunias by introducing a pigment-producing gene. However, instead of intensifying the color, the gene suppressed it, resulting in flowers with mixed white and purple colors or completely white. It took more than a decade and many significa nt experimental results to learn the mechanism of how RNAi works, and discover the power of RNAi. This work led to Drs. Fire and Mello winning a Nobel Prize in 2006. Their award-winning work, and that of countless other scientists, has opened many new areas of research in human, animal, and plant health.

Researchers around the world are discovering new ways that RNAi can be valuable to human, animal and plant health. In agriculture, RNAi provides a way to control pests and diseases and introduce novel plant traits. RNAi is an important technology with great promise to address pest mitigation needs in US agriculture while presenting an overwhelmingly desirable safety profile particularly in comparison to conventional pesticides. RNA based products will also have increased specificity, since they will be designed to be directed at known pest gene targets . Examples include nicotine -free tobacco, non -allergenic peanuts, decaffeinated coffee, and nutrient fortified corn among many others. At Monsanto, our researc hers are focused on tapping into the technology as a new way to use natural plant processes to improve oils in soybeans , increase the yield and quality potential of alfalfa, and as a new way of protecting plants from pests that attack the roots of corn plants underground.

Insect-resistant corn has been planted commercially in the U.S. since 2003, and accounted for over half the 2010 corn crop (Brookes and Barfoot 2012). Replacing or reducing chemical insecticides with insect -protected biotechnology plants has been shown to reduce the large volume of chemicals used against corn insect pests. As a result, in the period from 1996 to 2008, the overall amount of chemicals applied by famers to control these insects has fallen nearly 77 percent (Brookes and Barfoot 2010). In 2008 alone, the annual reduction in the volume of

applied insecticide active ingredient in corn was almost 4 million kilograms (Brookes and Barfoot 2010).

The corn event from Monsanto, MON 87411, builds on the current and safe biotechnology products planted by farmers today by adding a RNA component that only participates in the naturally occurring RNAi pathway in the corn rootworm (CRW) insect pest. MON 87411 will provide to the grower the important benefits of reducing the need for chemical insecticides, improving worker safety, increasing our corn production to feed an increasing population, and reducing the water footprint of agriculture on our land (CTIC 2011; Hurley et al. 2009; Towery and Werblow 2010).

2. Background

Corn (*Zea mays* L.) is the largest crop grown in the U.S. in terms of acreage planted and net value. In 2012, corn was planted on over 97 million acres and grain harvested from 87.4 million acres (USDA-NASS 2013b). Average yields in the previous five years ranged from 147 bushels per acre (bu/A) (2011) to 165 bu/A (2009) and were valued between $46.7 billion (2009) and $76.9 billion (2011) (USDA-NASS 2013a). The most damaging root-feeding pests of corn in the major U.S. corn growing regions are larvae of the corn rootworm complex (CRW: *Diabrotica* ssp., Coleoptera; Chrysomelidae) (Chandler et al. 2008). Corn rootworms can cause serious injury and yield losses and have been estimated to result in annual yield losses and control costs that exceed $1 billion (Metcalf 1986).

In February 2003, the EPA determined it was in the public interest to approve the first *Bacillus thuringiensis* (*Bt*) toxin targeting corn rootworm: Monsanto's Cry3Bb1 protein (event MON 863 (YieldGard Rootworm, EPA Reg. No. 524-528)) and the genetic material (Vector ZMIR13L) necessary for its production.

In December, 2005, EPA determined the benefit of MON 88017 was in the public interest and approved it for registration (EPA Reg. No. 524-551). The Cry3Bb1 toxin expressed in MON 88017 is equivalent (99.8% similar) to that in MON 863. The primary difference between the hybrids is that MON 88017 also expresses the CP4 EPSPS protein for resistance to glyphosate (Roundup®) based herbicides.

MON 87411 also expresses the Cry3Bb1 protein to control CRW pests and CP4 EPSPS protein for resistance to glyphosate (Roundup®) based herbicides. The Cry3Bb1 protein present in MON 87411 has over 99% amino acid identity to the Cry3Bb1 protein produced in MON 863 and the deduced amino acid sequence is identical to that produced from the expression cassette present in MON 88017. On March 31, 2004, U.S. EPA established an exemption from the requirement of a tolerance for residues of the plant-incorporated protectant Cry3Bb1 in corn (40 CFR § 174.518, revised and redesignated from § 180.1214, effective July 24, 2007). U.S. EPA

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also completed safety reviews of Cry3Bb1 in 2010 for its Biopesticide Registration Action Document for MON 863 (originally registered February 24, 2003) and MON 88017 (originally registered December 15, 2005).

MON 87411 builds upon the current *Bt* protein-based mode-of-action (MOA) for CRW control by the addition of a new RNA-mediated MOA that offers enhanced control of target insect pests and prolonged durability of existing *Bt* technologies designed to control CRW. MON 87411 contains a suppression cassette that expresses an inverted repeat sequence designed to match the sequence of western corn rootworm (WCR; *Diabrotica virgifera virgifera*). The expression of the suppression cassette results in the formation of a double-stranded RNA (dsRNA) transcript containing a 240 bp fragment of the WCR *Snf7* gene (DvSnf7). Upon consumption, the plant-produced dsRNA in MON 87411 is recognized by the CRW's naturally occurring endogenous RNA interference (RNAi) machinery, resulting in down-regulation of the targeted DvSnf7 gene leading to CRW mortality.

RNA-mediated gene suppression is a naturally-occurring, ubiquitous process in eukaryotes, including plants and animals that are consumed as food and feed. This suppression is responsible for several common characteristics of conventional crops including soybean seed coat color (Tuteja et al. 2004) and corn stalk color (Della Vedova et al. 2005), and has also been utilized in biotechnology-derived crops already approved for cultivation (Ivashuta et al. 2009; Parrott et al. 2010; Petrick et al. 2013). There are also RNAs present in the diet resulting from consumption of virally infected foods such as kidney bean, pepper, and barley (Fukuhara et al. 2005). As humans and livestock commonly consume RNA from different dietary sources, there is an established history of safe exposure to a full range of RNA sizes and sequences. In fact, several thousand naturally-occurring RNAs have been identified in rice that share a high degree of sequence similarity to the human genome and to several livestock genomes (Heisel et al. 2008), and many endogenous rice RNAs have been found to be complementary to mouse (*Mus musculus*), pig (*Sus scrofa*), cow (*Bos taurus*), and chicken (*Gallus gallus*) genes (Ivashuta et al. 2009). Additionally, a subset of RNAs in rice grain have been shown to have 100% exact sequence matches to critical transcripts in humans with no adverse effects upon consumption (Ivashuta et al. 2009). RNAs, regardless of size, sequence, or source, thus have a history of safe consumption in humans and livestock.

RNA-based products are an important and familiar technology with great promise to address pest mitigation needs in US agriculture while presenting an overwhelmingly desirable safety profile, particularly in comparison to conventional pesticides, that furthers EPA's mission to protect human health and the environment. This is in part due to RNA-based products having increased potential for specificity, because they can be designed to target only the desired pest genes of interest (Bachman et al. 2013; Whyard et al. 2009). In addition, the very large body of experience with human consumption of and environmental exposure to RNA has been acknowledged and relied on by EPA in a variety of regulatory decisions, including exposures from dsRNA originating from RNA-containing plant viruses (Petrick et al. 2013). The EPA has also acknowledged the dietary safety of nucleic acids by granting an exemption from the requirement of a tolerance for nucleic acids and residues that are part of plant incorporated protectants (PIP) (40 CFR 174.507, redesignated from § 174.475). Furthermore, in the case of non-pesticidal RNA, the FDA has determined nucleic acids are Generally Recognized as Safe

(GRAS) (U.S. FDA 1992) and this GRAS status has recently been relied on by USDA in regulatory decisions for deregulation of RNA-based products.

As of 2013, there were four *Bt* plant-incorporated protectants registered for control of CRW. EPA's review of monit oring data from 2007 to 2010 led EPA to conclude there was a general trend of reduced susceptibility of CRW to Cry3Bb1 with reports of heavy CRW damage in Cry3Bb1 fields recorded in at least 23 counties in the Corn Belt (reviewed in BPPD IRM Team Review, October 11, 2012). The development of CRW resistance is likely the result of a number of factors that increase the selection pressure on the protein, most notably the continuous planting of corn-on-corn with Cry3Bb1.

To preserve the established and highly valued current *Bt*-technology benefits, additional MOAs against CRW will be highly impactful to preserve their associated reduced need for insecticides and improvements in worker safety, increased yield protection, and water conservation. MON 87411 will not be offered for commercial use as a stand -alone product, but will be combined, through traditional breeding methods, with other deregulated biotechnology -derived traits to support the increased availability of pyrami ded products (totaling 3 MOAs against CRW) to provide protection against both above-ground and below-ground corn pests.

3. Corn Event MON 87411 Public Interest Finding

The criteria for determining whether registration of a pesticide chemical is in the pub lic interest are set forth in a Federal Register Notice dated March 5, 1986 (51 Federal Register (FR) 7628). There is a presumption that registration of a pesticide chemical is in the public interest if one of the following criteria is met: (1) the use is for a minor crop; (2) the use is a replacement for another pesticide that is of continuing concern to the Agency; (3) the use is one for which an emergency exemption under section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) has be en granted (i.e., the basis for the exemption was lack of a registered alternative product); or (4) the use is against a pest of public health significance. Further, the Environmental Protection Agency (EPA) may determine that such a registration is in the public interest on the basis of the following criteria: (1) there is a need for the new chemical that is not being met by currently registered pesticides; (2) the new pesticide is comparatively less risky to health or the environment than currently regist ered pesticides; or (3) the benefits (including economic benefits) from the use of the new active ingredient exceed those of alternative registered pesticides and other available non-chemical techniques.

The registration of MON 87411 is in the public interest given enhanced control of CRW pests by the addition of a new RNA-mediated MOA and because it will preserve the benefits (including economic benefits) already found to be in the public interest for existing CRW *Bt* technologies as indicated by the following factors:

> **i. Need for additional PIP modes of action for CRW control in US corn production** . CRW monitoring data, unexpected damage reports, and academic reports of field resistance have been reviewed extensively under BPPD special review to consider and review scientific uncertainties associated with corn rootworm resistance (U.S. EPA 2011; 2012; 2013e; a; c; b). EPA recommended that appropriate remedial action plans b e developed for instances where CRW resistance to Cry3Bb1 has been confirmed (U.S.

EPA 2010a). In response to reports of unexpected damage to Cry3Bb1 single mode of action products Monsanto implemented a robust product performance investigation process initiated in the 2012 growing season, to address grower reports of une xpected CRW damage, document cases that exceed regulatory threshold and mitigate CRW populations on these fields through application of "Best Management Practices" (BMPs). These measures included instructions for growers to use alternate control measures for CRW including crop rotation (to a non-CRW host plant), use of a pyramided product or a non-Cry3Bb1 variety, and finally, but least recommended, the application of conventional (soil -applied and adulticide) insecticides. MON 87411 introduces a valuable new tool for managing CRW, including Cry3Bb1-resistant populations, with this new RNA-mediated MOA. MON 87411 will provide control of target insect pests and prolonged durability of existing *Bt* technologies designed to control CRW. MON 87411 will provi de benefits to growers similar to those obtained by use of existing CRW -protected corn hybrids, which include reduced need for insecticides and associated improvements in worker safety, increased yield protection, and water conservation (CTIC 2011; Hurley et al. 2009 ; Towery and Werblow 2010), and will help sustain these benefits for existing Bt CRW-protection technologies through prolonged durability.

ii. Environmental risk profile and safety for humans, animals and Non -Target Organisms. One of the benefits of RNA- technology is that it can achieve sequence -specific gene silencing in only those insects targeted (Bachman et al. 2013; Whyard et al. 2009). In its consideration of ecological testing and assessment during the FIFRA Scientific Advisory Panel (SAP) January 28, 2014, EPA concluded that RNA sequence -specific factors may increase target specificity and reduce the likelihood of unintended effects (U.S. EPA 2014). Thus, when used to suppress genes critical for the targeted insect's survival, RNA can be used as selective insect control products that greatly reduce the likelihood of adversely affecting beneficial non-target organisms (NTOs).

In the U.S., regulatory guidelines for non -target organism (NTO) testing and risk assessment of insect protected crops have been developed by the EPA and testing is conducted according to a tier-based system (U.S. EPA 2010b). Additionally, the EPA has convened several SAP meetings to make recommendations and provide guidance for NTO testing and risk assessment for agricultural products produced by methods of biotechnology (U.S. EPA 2001 ; 2002b; 2004a; 2010c; 2013d). Environmental risk assessments were previously conducted by USDA (USDA-APHIS 2013) and EPA (U.S. EPA 2010c) for Cry3Bb1 -containing corn products MON 863 and MON 88017 and indicated no direct or indirect impact to the environment. MON 87411 builds upon the current *Bt* protein-based MOA for CRW control by the addition of a new RNA -mediated MOA that offers enhanced control of target insect pests and prolonged durability of existing *Bt* technologies designed to control CRW.

According to the Food Quality Protection Act of 1996 , EPA must reassess all existing tolerances to be sure that they meet the standard of "reasonable certainty of no harm." The EPA is required to first consider those pesticides that pose the highest risk to humans. EPA has previously completed safety reviews of Cry3Bb1 in 2010 for its

Biopesticide Registration Action Document for MON 863 (originally registered February 24, 2003) and MON 88017 (originally registered December 15, 2005).

There is a history of safe consumption of the RNA molecules mediating gene suppression in plants, including those with homology to genes in humans and other animals (Ivashuta et al. 2009 ; Jensen et al. 2013). As part of their assessment on the scientific issues associated with the use of RNA -technology as a pesticide (U.S. EPA 2014) EPA stated that the primary route of exposure is through the oral ingestion of RNA PIPs in food plants. Dietary RNA is extensively degraded in the mammalian digestive system by a combination of stomach acid and ribonucleases that are likely to ensure that all structur al forms of RNA are degraded throughout the digestive process. Based on these conditions, EPA concluded that there was no convincing evidence that ingested RNA is absorbed from the mammalian gut in a form that causes physiologically relevant adverse effect s, and any evidence of dietary uptake in mammals is nominal and non -specific (U.S. EPA 2014).

Nucleic acids, including RNA, have been noted to "not raise safety concerns" by EPA and are presumed GRAS (generally recognized as safe) by the U.S. FDA in the case of non-pesticidal applications (U.S. FDA 1992). In 2001, U.S. EPA established an exemption from the requirement of a tolerance for residues of nucleic acids that are part of a plant -incorporated protectant (40 CFR 174.507, redesignated from § 174.475, effective April 25, 2007). In additi on, EPA has previously determined RNA based active ingredients like Coat Protein of Plum Pox Virus to have benefit and be in the public interest (U.S. EPA 2010d).

Given the positive environmental safety and health profile of MON 87411, this PIP is considered to offer substantial benefits, including enhanced control of CRW, which will provide crop protection without significant increased use of chemical pesticides needed to control CRW insect pests. The targeted control of insect pests by MON 87411 should contribute to protecting U.S. agriculture, as well as our managed landscapes and natural areas.

iii. Sustained environmental and economic benefits. To qualify for a positive public interest finding, the product must demonstrate advantages in terms of the need for the chemical and its comparative benefits, risks, and costs. Monsanto Company has submitted a public interest document and other supporting documents that present the potential benefits of MON 87411 (Master Record Identification Numbers (MRID Nos.) 49315101-49315123). EPA has reviewed the submitted documents, public comments, syndicated marketing research studies, and published information. The major proposed benefits of MON 87411 corn for CRW control are as follows:

- Preservation of the current *Bt*-technology as an effective pest management tool by adding one additional MOA to increase product durability

- Replacement of chemical insecticides when responding to greater than expe cted damage from CRW

- Practical benefits for growers by reducing input costs, time and labor
- Equivalent or greater efficacy than current *Bt*-based products
- Human health benefits from reduced exposure to conventional insecticide use and favorable safety profile of RNA
- Environmental benefits from a reduced risk to non -target organisms compared to broad spectrum chemical insecticides used to control CRW
- Yield benefits expected to at least equal current *Bt*-based products

In conclusion, MON 87411 offers an additional new mode of action for CRW control and will prolong the durability of existing CRW -protected corn hybrids, thereby helping to sustain important environmental, health and economic benefits of CRW-protected corn.

Based on the above factors, the use of MON 87411 CRW-protected corn is in the public interest because it builds upon current *Bt* protein-based CRW control technology by introducing a new MOA based on the natural process of RNA -mediated gene suppression that will prolong the durability of existing *Bt* technologies that are used to control CRW. MON 87411 will provide benefits to growers similar to those obtained by use of existing CRW -protected corn hybrids, which includes reduced use of insecticides, increased yield protection, water conservation, and increased worker safety (Rice 2003) relative to conventional CRW control methods. MON 87411 is also glyphosate tolerant and will continue to provide benefits associated with conservation t illage methods, including reduced soil erosion, reduced fuel and labor costs, improved air quality and conservation of soil moisture (CTIC 2011; Hurley et al. 2009 ; Towery and Werblow 2010).

4. Product Characterization for MON 87411

Management of insect pests typically employs multiple tactics, including cultural control, plant resistance, mechanical control, biological control, chemical control, and integrated pest management. Three CRW control methods have been used for decades: (1) crop rotation, (2) soil-applied insecticides to protect the roots (approximately 90% of the total CRW -treated acres), and (3) use of adulticides to control CRW adult beetles (approximately 10% of the total CRW - treated acres) (Levine and Oloumi-Sadeghi 1991). Historically, greater than 90% of the growers use soil -applied insecticides, applied at planting to control larvae, due to greater efficacy and ease of application. Also, crop rotation has been the primary method of controlling CRW (Levine and Oloumi-Sadeghi 1991).

Transgenic CRW -resistant corn has been planted commercially in the U.S. since 2003, and comprised over half the 2010 crop (Brookes and Barfoot 2012). Replacing or reducing chemical insecticides with insect-protected transgenic plants has been shown to reduce the overall volume of chemical insecticide used against the target pest. In the period from 1996 to 2008, the overall volume of active ingredients in insecticides applied for control of European corn borer and other lepidopteran pests (beginning in 1996) and corn rootworm (beginning in 2003) has fallen nearly 77 percent (Brookes and Barfoot 20 10). In 2008 alone, the annual reduction in the volume of

applied insecticide active ingredient in corn was almost 4 million kilograms (Brookes and Barfoot 2010).

a. Comparative Toxicity to Humans (MRID Nos. 49315101, 49315102, 493151105, 493151106, 493151107, 493151108, 49315111)

The same Cry3Bb1 protein as contained in MON 87411 has been previously determined to be safe and in the public interest (U.S. EPA 1996 ; 2004b), and is currently widely planted as MON 88017-containing commercial corn hybrids; the second MOA for CRW control, the natural process of RNA -mediated gene suppression, and associated production of DvSnf7 dsRNA, is the only difference from current commercially available corn containing MON 88017.

The human safety assessment for nucleic acids introduced into biotechnology -derived crops takes the following into consideration: (a) the extensive history of safe consumption of nucleic acids, including both short and long RNA molecules with 100% sequence identity to human transcripts (Ivashuta et al. 2009 ; Jensen et al. 2013), (b) "introduced nucleic acids [in biotech crops], in and of themselves, do not raise safety concerns" (U.S. FDA 1992), (c) all food allergens are proteins (U.S. FDA 1992), and thus RNA is not a food aller gen, (d) there are extensive physiological and biochemical barriers that limit the potential for uptake or activity of ingested nucleic acids (Petrick et al. 2013), and (e) there is an absence of evidence indicating that dietary consumption of RNA is associated with toxicity (Petrick et al. 2013) or allergenicity, which includes RNA molecules associated with RNA mediated gene regulation.

Extensive sequence -independent physiological and biochemical barriers are known to exist in humans and ot her mammals that limit the potential for uptake or activity of ingested nucleic acids (Juliano et al. 2009 ; O'Neill et al. 2011 ; Petrick et al. 2013). Furthermore, there is no evidence to suggest that dietary consumption of nucleic acids is associated with toxicity (Petrick et al. 2013; U.S. FDA 1992). The US FDA has stated that all food allergens are proteins (U.S. FDA 1992), whereas there is no evidence of allergenicity of dietary RNA in the peer -reviewed scientific literature. The lack of toxicity or allergenicity for ingested RNA would also extend to RNA molecules associated with RNA -mediated gene regulation. The RNAs derived from the gene suppression cass ette in MON 87411 are presumed safe based on an extensive history of safe consumption for dietary RNAs, as reviewed by Petrick and colleagues (Petrick et al. 2013).

Total nucleic acid consumption in the human diet for DNA and RNA is estimated to be 1 -2 grams per day (Suchner et al. 2000) which equates to an approximate maximal intake of 0.2 g/kg/day for a non -nursing infant (the highest consumer per body weight). Tissue specifi c expression studies demonstrated that MON 87411 DvSnf7 dsRNA was expressed at mean levels ranging from 0.091×10^{-3} µg/g fw (in grain) to 14.4×10^{-3} µg/g fw (in over season leaf at growth stage V14-R1). Anticipated human dietary exposure to DvSnf7 dsRNA is also very low (≤ 0.4 ng/kg body weight per day) relative to estimated total daily RNA intake.

Based on the ubiquitous nature of RNA -mediated gene regulation in a wide variety of consumed plant species, demonstration of the specificity of DvSnf7 suppr ession in CRW, the long history of safe consumption of RNA from a range of sources, and the apparent lack of toxicity or allergenicity of dietary RNA, the DvSnf7 dsRNA suppression sequence used in MON 87411 poses no observed or theoretical risks to humans or animals.

b. Potential for Adverse Environmental Effects (MRID Nos. 493151102, 493151122)

The two components of the insecticidal activity against CRW of MON 87411 are the Cry3Bb1 protein and the DvSnf7 dsRNA. The environmental safety of the Cry3Bb1 protein has been demonstrated in multiple environmental studies and assessed extensively as part of many previous EPA product registrations, *e.g.*, MON 863, EPA Reg. # 524-528; MON 863 × MON 810, EPA Reg. # 524-545; MON 88017, EPA Reg. # 524-551, MON 88017 × MON 810, EPA Reg. # 524-552 and MON 89034 × MON 88017, EPA Reg. # 524-576 among others (U.S. EPA 2010c).

Soil organisms may be exposed to DvSnf7 by contact with roots and with above ground plant residues or pollen reaching the soil. Assessment of the environmental fate of DvSnf7 and any other nucleic acid (DNA or RNA) from biotechnology derived crops should consider the amount of nucleic acids already in the environment from conventional sources. Thousands of tons of nucleic acids are released into the environment every year from conventional plant biomass (roots, leaves, pollen, etc.), in addition to the nucleic acid contribution of other decaying animal and microbial matter (Dale et al. 2002). It is unlikely that nucleic acids originating from biotechnology-derived plants will persist in soil or interact differently than nucleic acids from non-engineered organisms, or persist by being incorporated into microbes via soil uptake (Dale et al. 2001).

Results from an aerobic laboratory soil degradation study (Dubelman et al. 2014) (MRID No. 49315122) demonstrate that the RNA expressed in MON 87411 dissipated rapidly in representative agricultural soils (MO, IL and ND) that ranged in pH from 5.9 to 7.1, and contained significant proportions of clay (up to 36%) or organic matter (up to 5.1%). Using methodology consistent with the current EPA approach to assess environmental fate for other PIPs, soil extracts were analyzed using QuantiGene, a molecular method for quantification of nucleic acids, and by insect bioassay (corn rootworm feeding), a functional measure of the insecticidal activity. Results indicated a maximum DT50 of 29 hours and a maximum DT90 of 35 hours for DvSnf7. These data indicate that a RNA from MON 87411 degraded completely within approximately two days after application to soil, which is equivalent to the degradation for current *Bt* proteins (Reviewed in U.S. EPA (2002c; a; 2010b)).

c. Impact to Non-Target Organisms, Including Those Beneficial to Agriculture (MRID Nos. 49315102, 49315105, 49315112 – 49315121, 49315123)

MON 87411 provides high target pest specificity, thus reducing risk to NTOs, including threatened and endangered species. The *Cry3Bb1* gene present in MON 87411 codes for a Cry3Bb1 protein that is nearly identical to the Cry3Bb1 protein produced by MON 88017. Consequently, the Cry3Bb1 produced by MON 87411 is predicted to have equivalent functional activity to the Cry3Bb1 protein produced by MON 88017. The activity of two functionally equivalent Cry3Bb1 proteins has previously been evaluated by the U.S. EPA (i.e., MON 863 and MON 88017 that are >99.8% identical) and concluded to have no unacceptable adverse effects on NTOs, including threatened and endangered species and their designated critical habitats. Exposure concentrations for the original MON 863 studies were based upon ≥10X the maximum observed Cry3Bb1 expression in MON 863 pollen, the highest expressing tissue. Because the maximum Cry3Bb1 protein levels are higher in MON 863 compared to MON 87411, the studies

performed for the U.S. EPA registration o f MON 863 provide margins of safety that indicate the low likelihood for adverse effects to NTOs from the Cry3Bb1 protein produced by MON 87411.

RNA-technology can achieve sequence -specific gene suppression in sensitive insects (sensitive as defined by pos sessing RNA machinery and demonstrating quantifiable responses to environmental exposure to RNA) by feeding RNAs (Bachman et al. 2013 ; Whyard et al. 2009). Sequence-specific gene suppression combined with the capability to suppress genes critical for insect survival means that RNAs can be used to develop insect control products that selectively target economically important pest species and greatly reduce the likelihood of adversely affecting beneficial NTOs. With RNA -technology, even closely related species of the same genus can be selectively controlled. Whyard et al. (2009) demonstrated with RNAs targeting regions of genes with no shared 21 nucleotide sequence identity, that four *Drosophila* species could be selectively controlled. It is also apparent from the published literature, that effective RNA interference is only achieved when a sufficient amount of RNA is supplied to effectively suppress the target gene (Bolognesi et al. 2012 ; Burand and Hunter 2013 ; Miller et al. 2012 ; Whyard et al. 2009).

Along with sequence specificity, there exist additional barriers to the oral toxicity in NTOs. These include potential degradation of the RNA prior to ingestion, physical and biochemical barriers as well as the inherent sensitivity of the organism to inges ted RNA (Whyard et al. 2009). As summarized in a recent review by Huvenne and Smagghe (2010), insects display a wide range of sensitivity to ingested RNA, with the order Coleoptera demonstrating significantly greater sensitivity than other insect orders. The order Lepidoptera has demonstrated variable sensitivity (to ingested RNA and high concentrations are required to elicit a response in this order relative to Coleopterans (Huvenne and Smagghe 2010 ; Terenius et al. 2011). Beyond insects, terrestrial and aquatic non -target organisms, including verteb rates, possess a number of physical and biochemical barriers to RNA between exposure in the environment and the target tissue.

Prior to conducting the NTO hazard studies for the assessment of DvSnf7 dsRNA, MOA of DvSnf7 dsRNA (Bolognesi et al. 2012) (MRID No. 49315102), the spectrum of insecticidal activity (Bachman et al. 2013) (MRID No. 49315102), f ate in soil (Dubelman et al. 2014) (MRID No. 49315122) and the potential for a toxicological interaction between DvSnf7 dsRNA and the Cry3Bb1 protein were evaluated (MRID No. 49315120 -49315121). The spectrum of activity was evaluated using a hypothesis -based phylogenetic approach that established the relationship between biological activity and phylogenetic relatedness to CRW. The potential effects were investigated with 14 insect species, representing 10 families and 4 orders. Representative insects from the following orders were exposed to DvSnf 7 dsRNA in diet bioassays: Hemiptera (*Orius insidiosus*), Hymenoptera (*Nasonia vitripennis* and *Pediobius foveolatus*), Lepidoptera (*Spodoptera frugiperda, Helicoverpa zea* , *Ostrinia nubilalis* , and *Bombyx mori*) and Coleoptera (WCR, SCR; *Diabrotica undecimpunctata howardi* , CPB; *Leptinotarsa decemlineata, Tribolium castaneum, Coleomegilla maculata, Epilachna varivestis,* and *Poecilus chalcites)*. Additionally, several species closely related to the WCR that could not be tested were evaluated in an indir ect method whereby the ortholog to the WCR *Snf7* gene (DvSnf7) was cloned and corresponding RNAs were tested against WCR and CPB in 12-day diet bioassays; two model systems known to be sensitive to ingested RNA (Bachman et al. 2013) (MRID No. 49315102). *Snf7* orthologs of five chrysomelid species: striped cucumber beetle

(*Acalymma vittatum)* , bean leaf beetle (*Cerotoma trifurcata*), and black -margined loosestrife beetle (*Galerucella calamariensis*) from the subfamily Galer ucinae, and Klamath weed beetle (*Chrysolina quadrigemina*) and yellow margined leaf beetle (*Microtheca ochroloma*), from the subfamily Chrysomelinae, were evaluated in these bioassays. The results from these bioassays supported the hypothesis that biological activity of DvSnf7 dsRNA is correlated with phylogenetic relatedness to the WCR and that DvSnf7 dsRNA activity is highly specific. Activity was evident only in a closely related subset of beetles within the family Chrysomelidae, specifically the subfamily Galerucinae (Bachman et al. 2013) (MRID No. 49315102).

The scope of species tested was broadened by the addition of nontarget organisms tested in the hazard assessment. The potential for toxicity of the DvSnf7 dsRNA was evaluated with several species of beneficial invertebrates including predators, parasitoids and detritivores in sub -chronic or chronic tests. Test species included the lady beetle (*Coleomegilla maculata*) (MRID No.49315114), carabid beetle (*Poecilus chalcites*) (MRID No.49315119), insidious flower bug (*Orius insidiosus*) (MRID No.49315117), parasitic wasp (*Pediobius foveolatus*) (MRID No.49315115), larvae and adult honey bee (*Apis mellifera*) (MRID Nos. 49315112, 49315113), Collembola (*Folsomia candida*) (MRID No. 49315118) and earthworm (*Eisenia andrei*) (MRID No. 49315116). Survival and/or growth and developmental observations were examined in the lady beetle, carabid beetle, insidious flower bug, parasitic wasp and honey bee stu dies, survival and reproduction with *Collembola* and survival and biomass with earthworm. Tests were of sufficient duration and evaluated ecologically relevant endpoints to adequately assess the potential for adverse effects based on the MOA of DvSnf7 dsRNA and time to effect in sensitive species. The results from these nontarget organism studies were evaluated using the maximum expected exposure concentrations (MEEC) values derived from the MON 87411 expression study to calculate margins of exposure (MOE). MOEs were calculated based on the ratio of the no observed effect concentrations (NOECs) to the MEEC, and provide an additional margin of safety over MEECs derived using LC_{50} values. All of the MOEs calculated for the NTO species were > 10-fold the predicted exposure level. Therefore, DvSnf7 dsRNA is not likely to produce adverse effects on terrestrial beneficial invertebrate species at field exposure levels. This conclusion is in agreement with prior published literature which reported t hat the spectrum of activity for DvSnf7 dsRNA is narrow or there is a low potential for adverse non-target effects.

d. Adverse Effects to Threatened and Endangered Species (MRID Nos. 49315123)

Threatened and endangered species risk assessments were previously conducted by the EPA (U.S. EPA 2010c) for Cry3Bb1-containing corn products MON 863 and MON 88017, and indicated no direct or indirect effects to threatened or endangered species.

Because of the specificity of the DvSnf7 dsRNA for coleopteran species within the family Chrysomelidae, endangered species concerns for DvSnf 7 dsRNA were focused on the order Coleoptera. Currently, there are 18 threatened and endangered Coleoptera; however, none of these species are members of the family Chrysomelidae (USFWS 2013). Additionally, the listed threatened or endangered coleopteran species are not expected to in or near corn fields and many of the endangered and threatened coleopteran species occur in caves or aquatic habitats (U.S. EPA 2010c). A comprehensive evaluation of the potential impact of DvSnf7 dsRNA on threatened and endangered Coleoptera was conducted and d ue to the lack of proximity to corn

cultivation, lack of relevant exposure because of feeding ecology and the restricted activity of the DvSnf7 dsRNA, it is concluded that cultivation of MON 87411 is not likely to directly or indirectly adversely affect threatened and endangered species and their critical habitats.

e. Potential for Toxicological Interaction (MRID Nos. 49315120, 49315121)

To bridge to NTO studies that tested DvSnf7 dsRNA alone as well as the previously conducted NTO studies that support the existing Cry3Bb1 plant incorporated protectant (PIP) registrations (U.S. EPA 2010c), the potential for interaction between the DvSnf7 dsRNA and the Cry3Bb1 protein was evaluated in insect bioassays with two insect species (MRID Nos. 49315120, 49315121). Demonstrating no interaction (synergism) between two or more PIPs allows for their toxicity to be assessed independently.

Bioassays were performed with: (1) SCR that is sensitive to DvSnf7 dsRNA and the Cry3Bb1 protein (Bachman et al. 2013; Bolognesi et al. 2012) and (2) CPB that is closely related to WCR, sensitive to the Cry3Bb1 protein (Meissle and Romeis 2009) but not sensitive to DvSnf7 dsRNA (Bachman et al. 2013). The *in vitro* synthesized DvSnf7 dsRNA and *E. coli*-produced Cry3Bb1 protein used in these studies w ere shown to be functionally equivalent to the DvSnf7 dsRNA (MRID No. 49315106) and the Cry3Bb1 protein produced *in planta* (MRID No. 49315107).

The experimental approach to assess the potential for interactions between the DvSnf7 dsRNA and Cry3Bb1 protein with SCR followed an established approach (Tabashnik 1992). This approach can be effectively used to exam ine the potential for synergy between similar or dissimilar toxins. The potential for interaction between DvSnf7 and the Cry3Bb1 protein with SCR was evaluated by comparing concentration responses in the presence and absence of the RNA at a fixed sub -lethal concentration and vice -versa. SCR concentration responses to the Cry3Bb1 protein alone and in presence of a fixed sub -lethal concentration of the DvSnf7 dsRNA were nearly identical demonstrating the lack of a synergy in a sensitive insect species. (MRID NO. 49315120).

Additionally, the potential for interaction between DvSnf7 dsRNA and the Cry3Bb1 protein was examined in CPB (MRID No. 49315121). CPB demonstrated nearly identical concentration effect relationships to the Cry3Bb1 protein alone and to the C ry3Bb1 protein in the presence of a fixed concentration of the DvSnf7 dsRNA indicating no synergy between Cry3Bb1 protein and DvSnf7 dsRNA in CPB.

Demonstrating no synergism between the Cry3Bb1 protein and DvSnf7 dsRNA allows for their independent testing and assessment. The results from testing performed with DvSnf7 dsRNA and Cry3Bb1 demonstrate no adverse effects to NTOs and the lack of interaction between DvSnf7 dsRNA and Cry3Bb1 indicates that no unexpected adverse effects would occur to NTOs from exposure to MON 87411.

Conclusion

MON 87411 contains the Cry3Bb1 protein and DvSnf7 dsRNA to provide protection against CRW pests and the CP4 EPSPS protein to provide tolerance to glyphosate herbicides in corn. MON 87411 builds upon current *Bt* protein-based CRW control technology by introducing a new

MOA based on the advantages of RNA -technology that offer increased control of target insect pests and will prolong the durability of existing CRW-controlling *Bt* technologies.

A weight of evidence has been developed by Monsanto and shared with EPA demonstrating MON 87411 and/or RNA -technology poses no adverse risk based on a history of safety for humans, livestock, and the environment. As a nucleic acid RNA is neither toxic, nor allergenic, and significant physiological and biochemical barriers exist in humans and other organisms to limit the uptake of RNA. Nucleic acids, to include RNA, have been noted to "not raise safety concerns" by EPA with the established exemption from the requirement of a tolerance for residues of nucleic acids that are part of a plant -incorporated protectant (40 CFR 174.507, redesignated from § 174.475, effective April 25, 2007). In addition, d ue to the ubiquitous presence of RNA in the environment, many potential non -target organisms also have physical and physiological barriers to RNA uptake. This, along with the fact that the DvSnf7 dsRNA component of MON 87411 has been shown to achieve high taxonomic specificity, creates a narrow taxonomic activity spe ctrum and limits the potential for adverse effects in non -target organisms.

Taken together, these factors should address the criteria established in the Federal Register Notice dated March 5, 1986 (51 FR 7628) in terms of need, comparative risk issues, a nd comparative benefits. As such, the registration of MON 87411 can be expected to be in the public interest.

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